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Han Li-na, [No Value]; Zhou Jin, [No Value]; Schuringa, Jan Jacob; Vellenga, Edo

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Review article

Treatment strategies in acute myeloid leukemia

HAN Li-na, ZHOU Jin, Jan Jacob Schuringa and Edo Vellenga

Keywords: acute myeloid leukemia; leukemic stem cell; treatment; novel agents

Objective To summarize the risk stratification and current treatment strategies for acute myeloid leukemia (AML) and discuss the role of emerging novel agents that might be applied in future clinical trials.

Data sources The data in this article were collected from PubMed database with relevant English articles published from 1991 to 2009.

Study selection Articles regarding the risk stratification and therapeutic options of AML, as well as the characteristics of leukemic stem cells were selected.

Results AML is a heterogeneous disease with variable clinical outcome dependent on several prognostic factors, including age, cytogenetics and molecular markers. The advances in the understanding of AML pathogenesis and development will generate potential novel agents that might improve the treatment results of standard chemotherapy.

Conclusion Deeper insight into the multiple transforming events of AML may aid us in designing combinations of small molecule inhibitors based on the individual patient characteristics.

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Acute myeloid leukemia (AML) is characterized by an accumulation of primitive hematopoietic cells in the bone marrow compartment due to a differentiation defect in stem/progenitor cells, resulting in disruption of normal hematopoiesis. Currently AMLs are categorized according to the World Health Organization (WHO) classification that is especially based on chromosomal abnormalities. Four subgroups of AMLs are classified. The first group is characterized by recurrent genetic abnormalities of prognostic significance. The second group is AML with myelodysplasia-related changes and the third group is therapy-related myeloid neoplasms. For the AML not otherwise specified (NOS), the definition is based on morphological and cytochemical and immunophenotypic features, representing the FAB classification (Table).

RISK STRATIFICATION

Several clinical studies have shown that the treatment of AML is dependent on several prognostic factors, including age, cytogenetics, mutational status and intensity of post-remission therapy. Cytogenetic features are the most well-defined prognostic factors, dividing patients into 3 main groups categorized as favorable, intermediate, and unfavorable risk groups.^{1,2} The unfavorable risk group can be further divided based on the presence of a monosomy karyotype (Figure 1).³ The 5-year overall survival (OS) is approximately 55%, 40% and 10% in the favorable, intermediate, and unfavorable risk groups, respectively. The age of the patients is tightly correlated with the cytogenetic features.⁴ The percentage of favorable cytogenetics drops from 20% to 7% with increasing age, while the percentage of patients in the unfavorable risk group increases from around 20% to 50%.^{1,2,4-6} The relative high percentage of patients with high-risk features implies that the treatment outcome of

elderly AML patients is poor.

Although cytogenetic aberrations provide valuable information for AML diagnosis and prognosis, a proportion of AML patients within the same risk group show heterogeneous responses to treatment. Core binding factor (CBF) AML is cytogenetically defined by the presence of chromosomal translocation t(8;21) or inv(16), presenting with favorable clinical outcomes.⁷ Mutations in KIT in this subtype of AML conferred to a more unfavorable prognosis.^{8,9} Cytogenetically normal AMLs (CN AMLs) were identified in around 50% of the patients, representing as a large heterogeneous subgroup within the intermediate-risk group.^{1,2,10,11} Falini et al¹² have recently shown that the group of patients can be further sub-divided based on the nucleophosmin (NPM) gene mutation. Patients with a NPM mutation without Fms-related tyrosine kinase 3 gene-internal tandem duplications (FLT3-ITDs) have a favorable prognosis that is comparable to patients categorized into the favorable risk group. CN AML patients with CCAAT/enhancer binding protein α (CEBPA) gene mutations also showed better OS,¹¹ whereby some studies suggested that the favorable role of CEBPA mutations were mostly dependent on the absence of FLT3-ITD in CN AMLs.¹³

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Department of Hematology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands (Han LN, Schuringa JJ and Vellenga E)

Department of Hematology, the First Clinical College of Harbin Medical University, Harbin, Heilongjiang 150001, China (Han LN and Zhou J)

Correspondence to: Prof. Edo Vellenga, Department of Hematology, University Medical Center Groningen, P.O. Box 30001, 9700 RB, Groningen, Netherlands (Tel: 31-50-3612354. Fax: 31-50-3614862. Email: e.vellenga@int.umcg.nl)

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Table. World Health Organization (WHO) classification of AML

Description	Genes involved	AML cases (%)
AML with recurrent genetic abnormalities		
AML with t(8;21) (q22;q22)	RUNX1-RUNX1T1	5
AML with inv (16) (p13.1q22) or t (16;16) (p13.1q22)	CBFB-MYH11	5-8
APL with t(15;17) (q22;q12)	PML-RARA	5-8
AML with t(9;11) (p22;q23)	MLLT3-MLL	2
AML with t(6;9) (p23;q34)	DEK-NUP214	1-2
AML with inv (3) (q21;q26.2) or t(3;3) (q21;q26.2)	RPN1-EVI1	1-2
AML (megakaryoblastic) with t(1;22) (p13;q13)	RBM15-MKL1	<1
Provisional entity: AML with mutated NPM	-	27-35
Provisional entity: AML with mutated CEBPA	-	6-15
AML with myelodysplasia-related changes		
Therapy-related myeloid neoplasms	-	24-35
AML, not otherwise specified (NOS)	FAB	10-20
AML with minimal differentiation (M0)		<5
AML without maturation (M1)		5-10
AML with maturation (M2)		10
Acute myelomonocytic leukemia (M4)		5-10
Acute monoblastic/monocytic leukemia (M5)		<5
Acute erythroid leukemias (M6)		<5
Pure erythroid leukemia		-
Erythroleukemia, erythroid/myeloid		-
Acute megakaryoblastic leukemia (M7)		<5
Acute basophilic leukemia		<1
Acute panmyelosis with myelofibrosis		Rare
Myeloid sarcoma		-
Myeloid proliferation related to down syndrome		-
Transient abnormal myelopoiesis		-
Myeloid leukemia associated with down syndrome		-
Blastic plasmacytoid dendritic cell neoplasms		-

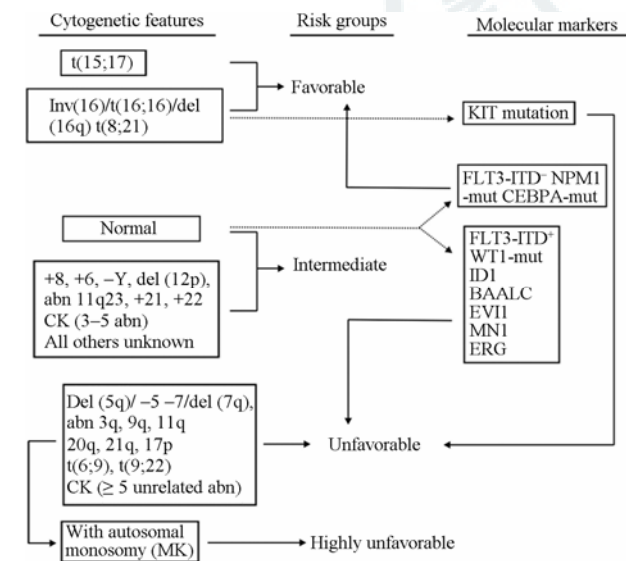


Figure 1. Cytogenetic and molecular classifications. CK: complex karyotype. MK: monosomy karyotype. abn: abnormal. FLT3-ITD: FLT3-internal tandem duplications. mut: mutant.

The AMLs with NPM mutant (mut) and wild type (wt) FLT3 can be further divided into subgroups based on the expression level of the erythroblastosis virus E26 oncogene homologue (ERG) gene. High level of ERG expression correlates with shorter event-free survival (EFS).¹⁴ In young patients with CN AML, low inhibitors of differentiation (ID1) expression levels predicted better disease-free survival (DFS) than those with high ID1 levels, with DFS even more improved in patients with

NPMmut/FLT3-ITD negative.¹⁵ In the study by the cancer and leukemia group B (CLGB), WT1 mutation was also an independent adverse prognostic factor in CN AML.¹⁶ However in the study by the German-Austrian AML study group, the negative impact of WT1 mutation was dependent on the presence of FLT3-ITDs in CN AML.¹⁷ Other genetic alterations including brain and acute leukemia, cytoplasmic (BAALC), EVI1 and Meningioma 1 (MN1) are also implicated in AML prognosis profiles, as negative indicators for OS.¹⁸⁻²⁰ Those molecular features on prognosis are summarized in Figure 1. In the future, high-throughput gene expression profiling will allow a genome-wide identification of subgroups within CN AML.²¹⁻²³ Recently, microRNA signature was also indicated in prognostically specific AML subgroups, associating with EFS.²⁴

Furthermore, fusion transcripts such as RUNX1-RUNX1T1 and CBFB-MYH11, as well as biomarkers, e.g. WT1, can be applied to monitor the minimal residual disease (MRD) and evaluate treatment response in AML.²⁵ Using MRD studies, risk-based therapies for AML can be designed to minimize toxicity and improve cure rates.

CURRENT TREATMENT STRATEGIES

Treatment for AML other than acute promyelocytic leukemia (APL)

Since APL is a unique subset of AML, with different treatment strategies and prognosis, it will be discussed separately. In this part, we focus on the treatment for AML other than APL.

Induction therapy

The 3+7 combination of the anthracycline daunorubicin (45 mg/m² or 60 mg/m² intravenously for 3 days) and cytarabine (Ara-C, 200 mg/m² continuously intravenous infusion for 7 days) has been the standard initial treatment for AML for 3 decades.²⁶ Approximately 50% to 75% of adults with AML achieve complete remission (CR) with this standard induction regimen.^{27,28} Attempts to improve CR rate by escalating the doses of cytarabine, substituting alternative anthracyclines idarubicin, addition of other cytotoxic agents, and others,^{11,29-32} have failed to show a significant survival advantage over the standard regimen, even though some approaches might improve EFS in certain subgroups. This treatment schedule was applied mostly for patients less than 60 years. Patients up to 60 years were treated with less intensive regimens. But a recent study has indicated that the treatment outcome of patients between 60 and 65 years can be improved by a more intense regimen especially for those in favorable risk group.³³ Remission induction chemotherapy has been proven to be important in elderly AML patients for improving clinical outcomes and is superior to reduced-dose chemotherapy and supportive treatment.^{34,35} Higher CR rates, lower early death rates and longer survival were achieved with intensive therapy compared with palliative therapy only. The clinical outcome

remains dismal in those patients with less tolerance to intensive chemotherapy, more preexisting comorbidities, and frequently unfavorable cytogenetics.⁴

Consolidation therapy

Consolidation treatment strategies have been applied in AML patients with CR to eliminate MRD, improve survival and increase cure rates. The treatment traditionally includes intensive consolidation chemotherapy, autologous stem cell transplantation (ASCT) or allogeneic stem cell transplantation (alloSCT) or combinations and low-dose maintenance therapy in the elderly patient group. Age and cytogenetic risk classification of the patients are important clinical parameters to determine the intensity of the consolidation therapy.

Variable chemotherapy regimens have been applied; also the numbers of courses vary between the different study groups.^{6,11,30} In general, 1–3 courses are applied after obtaining CR. The high-dose Ara-C (HiDAC) has been studied as standard consolidation chemotherapy especially in the USA.³⁶ The benefit was restricted to patients in favorable and intermediate risk groups, but not to patients in the unfavorable risk group.^{37,38} Elderly patients do not benefit from HiDAC, due to increased toxicity.

Hematopoietic stem cell (HSC) transplantation is performed with collected HSCs harvested from either the patient (autologous), or a human leukocyte antigen (HLA)-matched donor (allogeneic). A number of study groups have the policy that patients belonging to the favorable risk group should not be exposed to transplantation in view of the favorable prognosis.^{39–41}

ASCT is typically evaluated in patients belonging to the intermediate risk group in first remission (CR1), who do not have an available HLA-matched related donor. Some large clinical trials showed improvement in EFS, but not OS.^{42,43} In a recent clinical trial, no significant difference in OS was observed between chemotherapy and ASCT in young adults with CN AML.¹¹ Considering the higher mortality (<6%) of ASCT compared with consolidation chemotherapy, and improved efficacy of HiDAC, the advantage of ASCT is compromised compared to standard consolidation chemotherapy in this subgroup of patients.^{44,45}

AlloSCT is superior to ASCT for the presence of the immunological reaction referred to as graft-versus-leukemia (GVL) effect, in which the donated allogeneic cells recognize the recipients' leukemic cells as foreign, resulting in a better EFS. But it is associated with a higher risk of treatment-related mortality (10%–25%), which also dependent on the applied conditioning regimens.^{11,27,28} The recommendation of allogeneic transplantation must be based on the individual clinical characteristics, including age, risk stratification, type of donor match and prognosis. Significantly longer EFS and

OS have been revealed in a cohort of younger AML patients with normal karyotype.¹¹ Reasonably, patients with unfavorable cytogenetics and some patients with intermediate cytogenetics, except for those who are NPMmut/FLT3-ITD negative, are candidates for alloSCT.²⁸ A recent systematic review and meta-analysis demonstrated that alloSCT significantly improved EFS and OS in patients with intermediate and unfavorable risk, but not those with favorable risk, as compared with non-alloSCT.⁴⁶

Regarding the origin of the transplant, matched siblings should preferably be considered for alloSCT. Patients without matched siblings might receive a matched unrelated donor. The benefits were especially for patients ≤40 years in the setting of myeloablative conditioning.⁴⁷ Recently, reduced-intensity conditioning regimens have been introduced which makes it possible that older patients can also be treated with an alloSCT.⁴⁸ This provides the opportunity to transplant patients up to 65–70 years. To enlarge the application of alloSCT, umbilical cord blood (UCB) can be an option for patients without a matched donor. The advantages of UCB include rapid availability, greater tolerance of HLA disparity and lower incidence of severe graft-versus-host disease, as well as safety to donors, easy harvesting and less risk of transmitting infection.⁴⁹ But in adult patients, the major limitation is the low cell dose, which confers to the delayed engraftment, resulting in relatively higher infection rates. Double UCB transplantation can overcome the barrier of low cell dose and improve the hematological engraftment. As a summary, a schematic overview is shown for the therapeutic approaches in AML (Figure 2).

Maintenance therapy

Maintenance therapy is a standard care in APL, but has not convincingly been demonstrated to be effective in other subtypes of AMLs.²⁸ Various maintenance regimens have been explored but no advantages with regard to EFS or OS have been observed.^{50,51}

Treatment in relapsed disease

The majority of AML patients who gained first remission will relapse within 3 years of diagnosis.⁵² Treatment of relapsed AML is not satisfactory and chemotherapy alone is rarely curative. For those patients who can tolerate reinduction chemotherapy to obtain a second remission can proceed with ASCT or alloSCT. The most important factor to predict success for a second remission seems to be the duration of first CR. If the initial CR was more than 12 months, the possibility for a second CR could be 40% to 50%, which dropped till 10% to 20% if the initial CR was less than 12 months.^{53–56} The 3+7 combination and high-dose cytarabine have been suggested as effective reinduction regimens.^{45,57} AlloSCT could be the preferable treatment for most relapsed patients, especially for those who achieve a second remission, due to its potent anti-leukemic effects. For patients who can not

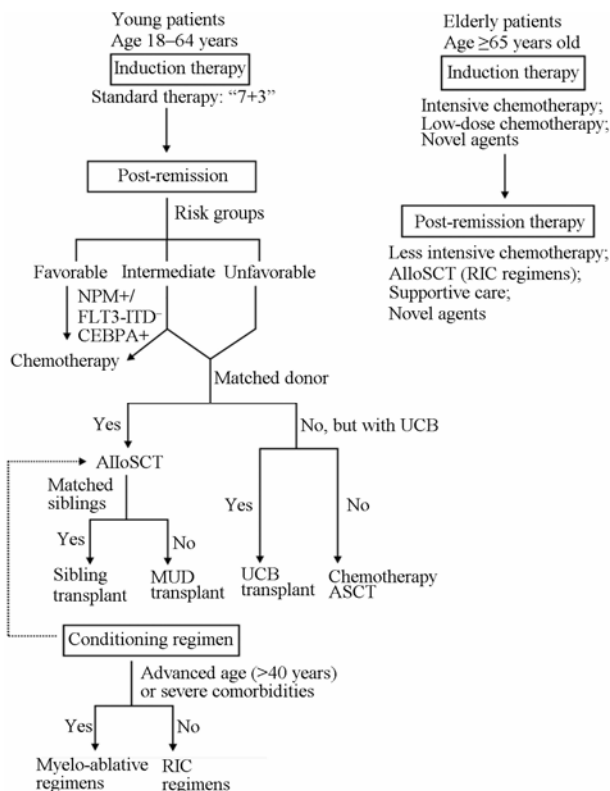


Figure 2. Schematic overview of AML treatment strategies. DNR: daunorubicin. NPM: nucleophosmin. CEBPA: CCAAT/enhancer binding protein α . FLT3-ITD: FLT3-internal tandem duplications. AlloSCT: allogeneic transplantation. ASCT: autologous transplantation. MUD: matched unrelated donor. UCB: umbilical cord blood. HLA: human leucocyte antigen. RIC: reduced-intensity conditioning.

tolerate a conventional conditioning regimen, nonmyeloablative and reduced-intensity allogeneic transplantation seem effective.⁵⁸ ASCT appears to be an option for AML in the second remission without available donors for allogeneic transplantation, with some possibilities of cure.⁵⁹ A 10-year survival rate of 32% was reported in patients in second remission with favorable and intermediate cytogenetics.⁶⁰

Treatment for acute promyelocytic leukemia

APL is a unique subset of AML characterized by the t(15;17) translocation which results in the PML-RAR fusion gene transcript.^{61,62} The main prognostic factors in APL are white blood cell (WBC) and platelet counts pretreatment. A predictive model based on WBC and platelet counts classified patients into 3 risk groups: low-risk group (WBC count $\leq 10 \times 10^9/L$ and platelet count $> 40 \times 10^9/L$), intermediate-risk group (WBC count $\leq 10 \times 10^9/L$ and platelet count $\leq 40 \times 10^9/L$) and high-risk group (WBC count $> 10 \times 10^9/L$).^{63,64} Risk-adapted treatment protocols have been designed based on relevant characteristics.

The current standard approach with all-trans retinoic acid (ATRA) plus anthracycline chemotherapy significantly improved the clinical outcome of newly diagnosed APL patients, with CR rate of approximately 90% and 5-year

cumulative DFS of 84%.⁶⁵⁻⁶⁸ The addition of cytarabine in this regimen can benefit high-risk patients, but not low- and intermediate-risk groups.⁶⁹ Alternatively, the benefit of ATRA/arsenic trioxide (ATO) combination therapy in newly diagnosed APL was demonstrated in a recent study from Chinese institutes. With a median follow-up of 70 months, 94% of patients achieved CR, and 5-year EFS and OS were 89% and 92%.⁷⁰ This combination therapy was also reported from the M.D. Anderson Cancer Center, with gemtuzumab ozogamicin (GO) added to high-risk patients.⁷¹ These analyses suggested that ATRA combining with ATO induction therapy without chemotherapeutic reagents could be safely and effectively applied in *de novo* APL and would require additional clinical studies. Recently the efficacy and safety of ATO as a single agent was demonstrated in newly diagnosed pediatric APL.⁷²

Consolidation therapy with at least 2 further cycles of anthracycline-based chemotherapy after induction allows the achievement of molecular remission of patients.⁷³ The superiority of adding ATRA into consolidation was shown in GIMEMA and PETHEMA cooperative groups.^{74,75} The role of ATO in postinduction therapy was also identified with high antileukemic activity.^{76,77} Molecular assessment by real time polymerase chain reaction (RT-PCR) of promyelocytic leukemia-retinoic acid receptor alpha (PML-RARA) fusion gene was recommended at the end of consolidation chemotherapy to determine the relapse risk in APL patients.^{78,79} For patients achieving molecular remission after consolidation therapy, ATRA-based maintenance therapy for 2 years was recommended to eliminate the MRD and decrease the relapse rate.^{68,80}

Treatment for central nervous system leukemia (CNSL) in AML

Extramedullary infiltration (EMI) that includes tumor nodules (myeloid or granulocytic sarcoma), skin infiltration (leukemia cutis), meningeal infiltration, gingival infiltration, hepatosplenomegaly or central nervous system leukemia (CNSL), occurs in around 30% of patients with AML.^{81,82} EMI has been reported to be more common in myelomonoblastic and monoblastic subtypes of AML (FAB M4 and M5) and AML with favorable cytogenetics, e.g. t(8;21) and inv(16). The frequency of EMI in APL is reported to be between 3%–8%.⁸³ The prognostic significance of EMI is controversial. Some studies report that EMI confers a poor prognosis, particularly in pediatric AML patients or patients with t(8;21).^{81,84} With current treatment advances, extramedullary relapses outside the CNS have become exceedingly rare; however, CNS relapse still occurs in 2%–4% of patients with AML.^{85,86} But due to the low frequency of CNS involvement in AML, controversy exists in prophylactic therapy for CNSL. CNS-directed treatment protocols of *de novo* AML patients include cranial irradiation, intrathecal treatment and systemic chemotherapy.⁸⁵ Since contemporary treatment protocols include high-dose cytarabine or other drugs that penetrate

readily into the CNS, cranial irradiation is not necessary. For the intrathecal treatment, cytarabine, especially the liposomal cytarabine that can maintain a therapeutic level of cytarabine in cerebrospinal fluid, has been the preferred drug in AML by reducing the CNS relapse rates.^{87,88} It remains unclear for the effect of systemic chemotherapy on CNS prophylaxis in AML. And less is known about the best treatment for patients with isolated CNS relapse of AML. In summary, the strategy for CNS disease control still remains to be optimized. Further studies on large cohorts of AML patients need to be performed.

CURRENT CHALLENGES OF AML TREATMENT

Although many improvements have been achieved in the treatment of AML patients, clinical outcomes are still not satisfactory, which are strongly dependent on the risk group. Emerging studies consider that leukemic stem cells (LSCs) initiate and maintain AML, and their quiescent state contributes to resistance of the conventional chemotherapy. Recent studies have demonstrated that leukemic transformation is a multistep process.⁸⁹ Multiple acquired genetic changes occurred to convert normal HSC to LSC which was characterized by enhanced self-renewal, increased proliferation, impaired differentiation and apoptosis, resulting in accumulation of immature AML blasts.⁹⁰ Understanding AML pathogenesis and biological properties of LSCs will be helpful to develop LSC-targeted therapies, and finally cure the disease since so far no further improvement has been obtained by more dose-intensification of chemotherapy. The following section will focus on novel agents targeting the unique properties of LSCs in AML.

Targeting LSC by unique membrane antigens

Theoretically, the plasma membrane molecules preferentially expressed in subsets of AML LSCs, such as CD123 (IL-3R α), CD33, C-type lectin-like molecule-1 (CLL-1), CD96 and CD47,⁹¹⁻⁹⁴ would be ideal LSC targets. A neutralizing antibody (7G3) targeting CD123 impaired the homing and engraftment ability of AML LSCs in NOD/SCID mice and inhibited IL-3-induced intracellular signaling of AML CD34⁺CD38⁻ cells in vitro.⁹⁵ This antibody is most effective when the leukemic burden is low, suggesting it can be applicable during remission induction after chemotherapy. A humanized anti-CD33 mAb, GO (Mylotarg) was approved by the FDA for treatment of CD33⁺ AML in the first relapse in elderly patients, who were not tolerable for chemotherapy.⁹⁶ In phase II clinical trials, about 30% of elderly patients in the first relapse can achieve CR by using GO monotherapy.^{97,98} However, CD33 is also expressed on normal HSCs, which may explain the prolonged cytopenia following treatment with GO in some AML cases.⁹⁹ So far the most prominent effect of GO has been observed in patients with relapsed APL, which might be due to a homogeneous CD33 expression pattern with increased antigen density of CD33 in APL.¹⁰⁰

Preclinical studies have recently also shown that CD47 might be therapeutic target for AML stem cells.^{101,102} It was observed that treatment with anti-CD47 antibody can enable phagocytosis of AML LSCs and inhibit *in vivo* engraftment, without affecting their normal counterparts.

Molecular targeting of activated tyrosine kinases

FLT3 inhibitors

Approximately 30% of AML cases contain FLT3-ITDs, which is a strong negative prognostic factor in AML, especially in conjunction with NPM mutations. Various small molecule kinase inhibitors against FLT3 activation have been developed and evaluated in AML patients, including PKC-412 (midostaurin), CEP-701 (lestaurtinib), SU-11248 (sunitinib), MLN-518 (tandutinib), KW-2449, SU-5416 and sorafenib.¹⁰³⁻¹⁰⁹ In most of those clinical trials, FLT3 inhibitors were evaluated in relapsed or refractory AML patients. Generally, FLT3 inhibitors showed only modest clinical efficacy, with blasts reduction as the most dominant effect. But CR is rarely observed. Recent studies revealed the importance of unique and sustained inhibition of FLT3, and developed a new compound, AC220, a uniquely potent and selective FLT3 inhibitor with desirable safety and pharmacokinetic profile.^{110,111} A recent study demonstrated that the sensitivity towards FLT3 inhibitors was enhanced by a CXCR4 antagonist by abrogating the protective signals from stromal cells.¹¹² It is likely that single use of FLT3 inhibitors will be of limited value especially from the perspective that leukemic transformation depends on at least two transforming events.⁸⁹ Therefore, the effects of FLT3 inhibitor should be considered in the setting of additional chemotherapy.

KIT inhibitors

KIT mutations occur in more than 50% of patients with CBF AML, particularly in FAB M2 subtype, associating with inferior clinical outcomes.¹¹³ Imatinib mesylate (IM) inhibits KIT kinase activity, but it is limited to wt KIT and juxtamembrane domain mutations.¹¹⁴ KIT activation loop mutations are frequently noticed in AML samples that are resistant to imatinib.^{115,116} Dasatinib was designed as a dual ABL-/SRC-family kinase inhibitor.¹¹⁷ It was demonstrated that dasatinib can inhibit the kinase activity of both wt and mut KIT isoforms, with differential potency against mutations involving codon 816 and 822, suggesting it may benefit AML patients with KIT activation loop mutations.^{118,119} A recent report showed dasatinib treatment together with chemotherapy can induce long-term hematologic and molecular remission in a patient with AML harboring KIT D816V mutation.¹²⁰ A potent KIT inhibitor, APcK110 showed inhibitory effects on AML blast colony-forming cell proliferation without affecting normal bone marrow cells.¹²¹ Those inhibitors could provide therapeutic advantages in AML, and their efficacy needs to be verified in clinical trials.

Farnesyl transferase inhibitors

Farnesyl transferase inhibitors (FTIs) have been developed to block constitutive RAS activation which

requires farnesyl transferase for localization to the plasma membrane.¹²² Tipifarnib, one of the FTIs that has been applied in phase II clinical trials with CR rates of 4% in refractory or relapsed AML patients and 14% in unfavorable-risk elderly patients.¹²³⁻¹²⁵ Prolonged DFS in a subgroup of unfavorable-risk patients was observed when tipifarnib was applied as maintenance therapy in CR1.¹²⁵ Meanwhile, despite the initial attempts of FTIs to target RAS mutations, no correlation between RAS mutations and response of FTIs can be demonstrated in clinical trials.¹²⁶ Furthermore, NRAS and KRAS, the most dominant isoforms involved in AML, can be alternatively geranylgeranylated to escape functional inactivation by FTIs.¹²² In summary, RAS mutations remain unattractive as targets in AML therapeutic interventions.

Epigenetic modulators

Epigenetics is defined as heritable changes in gene expression that are not accompanied by alterations in primary DNA sequence.¹²⁷ Epigenetic modifiers targeting DNA methylation and histone acetylation (histone deacetylase, HDAC) have been developed to release the gene repression in AML. Various HDAC inhibitors, including e.g. romidepsin, valproic acid, butyrate, MGCD0103, and hydroxamic acid, did not show satisfactory results as single agents.¹²⁸⁻¹³² DNA methyltransferase (DNMT) inhibitor azacitidine significantly improved OS as compared to conventional care, especially in patients with AML and high-risk myelodysplastic syndromes (MDS) with chromosome 5 and 7 abnormalities.^{133,134} Recently, the superiority of azacitidine was demonstrated in AML patients with low marrow blast count (20%–30%).¹³⁵ Dual targeting of both HDAC and DNMT was hypothesized to be a rational approach to AML treatment.¹³⁰ However, this concept was challenged in a recent study in which reversal of promoter methylation was observed in both clinical responders and non-responders.¹³⁶

Inducing apoptosis

A relative insensitivity to apoptotic stimuli results in survival advantages of the leukemic cells. Nuclear factor-kappa B (NF- κ B) is highly activated in AML leukemic cells but not in normal CD34⁺ cells, suggesting inhibition NF- κ B might induce LSC-specific apoptosis.^{137,138} MG-132, an inhibitor of NF- κ B, showed rapid induction of apoptosis exclusively in LSCs, but not in normal CD34⁺ cells.¹³⁹ The role of BCL-2, a member of anti-apoptotic family, was also identified in AML pathogenesis. *In vitro*, down-regulation of antisense oligonucleotides increased sensitivity of AML cell lines to chemotherapy.¹⁴⁰ The efficacy of BCL-2 antisense oligonucleotide (G3139) has been proven in phase I clinical trials combining with chemotherapy.^{141,142}

Release of LSCs from their microenvironment

Both normal and leukemic stem cells reside in a bone marrow niche, a specialized microenvironment.¹⁴³⁻¹⁴⁵

Jagged-Notch, Tie2-Angiopoietin-1 and CXCR4-SDF1/CXCL12 axis, as well as RAC signal transduction pathways have been demonstrated to be important in regulating the interaction of stem cells with their niche.^{144,146} Homing to the microenvironment is important for sustaining LSC survival. CD44, a transmembrane glycoprotein mediating cell-cell and cell-extracellular matrix interactions, was identified as a key regulator of AML LSCs.¹⁴⁷ Targeting CD44 with a monoclonal antibody (H90) eradicated AML LSCs by blocking the homing ability and altering the stem cell fate. In addition, in a mouse model of CML, BCR-ABL-positive LSCs are more heavily dependent on CD44 for homing and engraftment as compared to normal HSCs.¹⁴⁸ Those studies indicated that CD44 blockade can be a LSC-specific approach in leukemia treatment. CXCR4 was highly expressed in AML blasts, predicting shorter survival independently.¹⁴⁹ AMD3100, a small molecule inhibitor acting as CXCR4 antagonist, inhibited the transmigration and colony formation of AML blasts.¹⁵⁰ Administration of AMD3100 enhanced mobilization of leukemic blasts, increased sensitivity of chemotherapy and improved OS in mouse model.¹⁵¹ In the past two decades, hematopoietic growth factors have been applied as priming agents to drive leukemic cells entry into cell cycle and increase susceptibility to chemotherapy.^{30,152-154} Some studies showed that younger AML patients with intermediate-risk cytogenetics might benefit from G-CSF or GM-CSF priming.^{30,154} A recent study demonstrated that G-CSF-mediated stem cell mobilization was CXCR4-dependent.¹⁵⁵ It suggested that release of the leukemic cells from their protective environment was involved in the mechanisms of growth factor priming. The usefulness of CXCR4 antagonists in combination of chemotherapy may be provided in future clinical trials in AML patients.

Targeting ATP-binding cassette (ABC) transporters

Another method to target LSC is to circumvent the problem of conventional chemotherapeutic drug resistance, due to high expression levels of ATP-dependent drug transporters in AML LSCs.¹⁵⁶ The ABC transporter family comprises 49 functionally distinct transmembrane proteins, classified as 7 subfamilies (ABCA through ABCG).¹⁵⁶ The first and second generations of MDR1 inhibitors, such as verapamil and PSC-833 (valsopodar) failed to achieve promising results.¹⁵⁷⁻¹⁵⁹ This may be due to multiple ABC transporters other than MDR1 itself being involved in drug efflux. Some studies demonstrated that the ABCG2 transporter was predominant in HSCs instead of MDR1.¹⁶⁰ Apparently, only modulating MDR1 is not enough in AML treatment. The third generation of MDR modulators with multiple functions are under development and evaluation.¹⁵⁶ It should be noted that redundancy of ABC transporter expression not only exists in normal or leukemic stem cells, but also has relevant functions in liver and kidney, which dictate therefore the pharmacokinetics of the drug and increased toxicity.¹⁶¹

Other new agents

Anti-angiogenesis agents are potential approaches in AML based on evidences that bone marrow biopsies from AML patients demonstrate increased neovascularization, which was associated with poor prognosis.¹⁶² Elevated level of vascular endothelial growth factor (VEGF) was identified in AML to promote disease progression.¹⁶³ SU5416 showed clinical activity in a phase II trial of AML patients who did not tolerate chemotherapy.¹⁰⁴ Other agents including thalidomide, a putative inhibitor of angiogenesis, and bevacizumab, an anti-VEGF antibody were also evaluated in clinical trials.^{164,165} So far no significant improvement in OS has been observed in elderly AML patients that have been treated chemotherapy alone. In the coming years the effects of lenalodome in conjunction with chemotherapy will be tested. In patients with unfavorable-risk or refractory/relapsed AML, second generation nucleoside analogues such as clofarabine and troxacitabine showed clinical activity by inhibiting DNA repair and activating apoptotic pathway.^{166,167} New agents targeting translation factors and mitosis are currently under investigation.^{168,169}

CONCLUSION

Clinical outcomes with standard therapies strongly depend on the risk group, especially the outcomes for elderly patients. Probably this is related to a difference in cell biology between the older and younger leukemic stem cells. A recent microarray study in younger and elderly AML patients demonstrated that p16INK4A expression was down-regulated with increasing age in the intermediate and unfavorable risk group, which was in contrast to the elevated expression level of p16INK4A with physiologic aging.¹⁷⁰ It is likely that new insights into the multiple transforming events of AML will lead to a better definition of the therapeutic targets. Ultimately by combining small molecule inhibitors that specifically target the genotype-specific mutations we should be able to treat AML more effectively.

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